PATENT COOPERATION TREATY

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To:
THERESA A. BROWN
SHERIDAN ROSS P.C.
1560 BROADWAY
SUITE 1200
DENVER, CO 80202-5141

Received:
Date: 1-3-02
Initial: AKD
Please Return to Janice

PCT SHERIDAN, ROSS

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	19 DEC 2001
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
4152-3-PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/19336	13 July 2000 (13.07.2000)	13 July 1999 (13.07.1999)

Applicant

BOLDER NIOTECHNOLOGY INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Jessica H. Roark
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4152-3-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/19336	13 July 2000 (13.07.2000)	13 July 1999 (13.07.1999)

International Patent Classification (IPC) or national classification and IPC

IPC(7): A61K 39/395, 39/00; C07K 16/00, 01/00 and US Cl.: 424/134.1, 185.1, 192.1; 435/69.7; 530/387.3, 351

Applicant

BOLDER NIOTECHNOLOGY INC.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 January 2001 (08.01.2001)	02 October 2001 (02.10.2001)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Jessica H. Roark Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

I. Basis of the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed.

☒ the description:
pages 1-62 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages 63-65, filed with the letter of 15 August 2001 (15.08.2001)

☒ the drawings:
pages 1, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/fig NONE

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 6-14,22,23 and 26-37

because:

☐ the said international application, or the said claim Nos. ____ relate to the following subject matter which does not require international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 6 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 6 is an improper multiple dependent claim as per PCT Rule 6.4(a).

☐ the claims, or said claims Nos. ____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 7-14, 22-23 and 26-37

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 3-5 and 17-20 and 25	YES
	Claims 1-2, 15-16, 21 and 24	NO
Inventive Step (IS)	Claims 17-20 and 25	YES
	Claims 1-5, 15-16, 21 and 24	NO
Industrial Applicability (IA)	Claims 1-5, 15-21 and 24-25	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Section I. Basis of the report, Item 5

The amendment of the claims, filed 15 August 2001 is objected to under PCT Article 34(2)(b) because it adds matter into the application that goes beyond the disclosure as orginally filed. The added matter which appears to be new is as follows:

The amendment filed 15 August 2001 does not point to a passage in the disclosure for the added matter. In claim 40, both the lower limitation of "two or more" and the negative limitation of "wherein the peptide linker is not Gly 3-7" appear to be added matter that goes beyond the disclosure as originally filed.

1. Claims 1-2 and 24 lack novelty under PCT Article 33(2) as being anticipated by US 5,650,150 (GILLIES).

US 5,650,150 teaches a fusion protein comprising a the cytokines IL-2, lymphotoxin, or GM-CSF joined either directly or via a linker sequence comprising a proteolytic cleavage site to an immunoglobulin domain (see entire document, especially claims 1, 3 and 7-11). In addition, a purified dimeric Ig fusion protein is taught, since the CH3-LT fusion protein forms dimers (e.g., column 8, especially lines 56-65), and can be purified (e.g., column 9, lines 25-43).

2. Claims 15-16 and 21 lack novelty under PCT Article 33(2) as being anticipated by US 5,073,627 (CURTIS et al.).

US 5,073,627 teaches a multimeric fusion protein comprising two or more members of the GH supergene family (GM-CSF and IL-3) joined with or without a peptide linker (see entire document, especially claim 1). In addition, a multimeric fusion protein wherein one of the members is GM-CSF is taught (e.g., claim 1).

3. Claim 2 and 24 lack novelty under PCT Article 33(2) as being anticipated by SHU et al.

SHU et al. teach a fusion protein comprising a first protein joined by a peptide linker to a fragment of an immunoglobulin domain, comprising the cytokine IL-2 linked via a GGGSGGG linker to the CH3 of an immunoglobulin heavy chain (see entire document, especially "Abstract". Purification of this dimeric Ig fusion protein is also taught (e.g. "Abstract" and Section 2.5).

4. Claims 15-16 and 21 lack novelty under PCT Article 33(2) as being anticipated by CURTIS et al.

CURTIS et al. teach a multimeric fusion protein comprising GM-CSF either linked directly or through a peptide linker to IL-3 (see entire document).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

5. Claim 2 lacks novelty under PCT Article 33(2) as being anticipated by US 5,723,125 (CHANG et al.).

US 5,723,125 teaches a fusion protein comprising a human interferon linked to an immunoglobulin Fc via a peptide linker comprising Gly and Ser (see entire document, e.g., "Abstract"). US 5,723,125 also teaches that the incorporation of a linker sequence is useful to avoid formation of neoantigens in the fusion protein (e.g., column 3, especially lines 22-35).

6. Claims 1-5 and 24 lack an inventive step under PCT Article 33(3) as being obvious over EITHER US 5,650,150 (GILLIES), OR SHU et al., OR US 5,723,125 in view of ROBINSON et al.

The claims are drawn to various peptide linker sequences used to link a first protein to an immunoglobulin domain.

US 5,650,150 has been discussed supra and teach a first protein linked to an immunoglobulin domain, either directly or with a linker comprising a proteolytic cleavage site, and the dimeric protein's purification.

SHU et al. or US 5,723,125 likewise have been discussed supra and teach a first protein linked via a peptide linker sequence to an immunoglobulin domain.

Neither US 5,650,150, SHU et al. nor US 5,723,125 teach the exact linkers recited.

ROBINSON et al. teach various linkers, and that different linkers comprising various amount and sequence compositions of Gly and Ser can be used for any given fusion construct in order to optimize the stability of single chain proteins (see entire document).

Given the teachings of the references in view of ROBINSON et al., it would have been obvious to the ordinary artisan at the time the invention was made to select for and optimize various linker sequences depending upon the first protein linked to the immunoglobulin domain. The ordinary artisan would have been motivated to optimize this linkage in order to obtain a stable fusion protein. Given the teachings of the references, the ordinary artisan would have had a reasonable expectation of success in producing any particular linkers comprising varying ratios and sequences of Gly and Ser. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

7. Claims 1-5, 15-21 and 24-25 appear to have industrial applicability as defined by PCT Article 33(4) since the fusion proteins of the instant invention could be used in the various methods of stimulating cell growth.

---------- NEW CITATIONS ----------

US 5,073,627 A (CURTIS et al.) 17 December 1997, (17-12-1997), see entire document, especially "Abstract" and "Claims".

US 5,650,150 A (GILLIES) 22 July 1997, (22-7-1997), see entire document, especially "Claims".

ROBINSON et al. Optimizing the stability of single-chain proteins by linker length and composition mutagenesis. Proc. Natl. Acad. Sci. USA. May 1998, Vol. 95, pages 5929-5934, see entire document, especially "Discussion".

1. A fusion protein comprising a soluble protein joined without an intervening peptide linker to an immunoglobulin (Ig) domain, wherein the soluble protein is selected from the group consisting of a growth factor, a cytokine that is not IL-10, and an active variant thereof, and wherein the immunoglobulin domain does not contain a variable region.

2. A fusion protein comprising a soluble protein joined at its carboxy-terminus to the amino terminus of an immunoglobulin domain, wherein the soluble protein is selected from the group consisting of a growth factor, a cytokine that is not interleukin-10 (IL-10) or an interferon, and an active variant thereof, wherein the immunoglobulin domain does not contain a variable region, and wherein the soluble protein and the immunoglobulin domain are joined by a peptide linker that is not AspProGlu or Ser.

3. The fusion protein of claim 2, wherein the peptide linker is SerGly.

4. The fusion protein of claim 2, wherein the peptide linker is $Ser(GlyGlySer)_n$, wherein n is 1 to 7.

5. The fusion protein of claim 2, wherein the peptide linker is Ser(GlyGlySer) or $Ser(GlyGlySer)_2$.

6. The fusion protein of any one of claims 1-5, wherein the Ig domain is selected from the group consisting of IgG-Fc, $IgG\text{-}C_H$ and $IgG\text{-}C_L$.

7. The fusion protein of any one of Claims 1-5, wherein the soluble protein is a member of the growth hormone (GH) supergene family.

8. The fusion protein of any one of Claims 1-5, wherein the soluble protein is granulocyte-colony stimulating factor (G-CSF).

9. The fusion protein of claim 8, wherein the fusion protein has an EC_{50} of less than about 300 ng/ml in a G-CSF-dependent cell assay.

10. The fusion protein of claim 8, wherein serine is substituted for cysteine-17 of G-CSF.

11. Canceled.

12. The fusion protein of any one of Claims 1-5, wherein the soluble protein is growth hormone (GH).

13. Canceled.

14. The fusion protein of any one of Claims 1-5, wherein the soluble protein is selected from the group consisting of granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-11 (IL-11), thrombopoietin (TPO), stem cell factor (SCF) and flt3 ligand.

15. A homomultimeric fusion protein comprising two or more copies of a member of the Growth Hormone (GH) supergene family joined without an intervening peptide linker.

16. A homomultimeric fusion protein comprising two or more copies of a member of the Growth Hormone (GH) supergene family joined by at least one peptide linker, wherein the member of the GH supergene family is selected from the group consisting of: growth hormone, prolactin, placental lactogen, thrombopoietin (TPO), interleukin(IL)-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-10, interleukin-11, interleukin-12 (p35 subunit), interleukin-13, interleukin-15, oncostatin M, ciliary neurotrophic factor, leukemia inhibitory factor, alpha interferon, beta interferon, gamma interferon, omega interferon, tau interferon, granulocyte-colony stimulating factor (G-

CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), cardiotrophin-1, macrophage colony stimulating factor, Stem Cell Factor and flt-3 ligand.

17. The homomultimeric fusion protein of any one of claims 15-16, wherein the member of the GH supergene family is granulocyte-colony stimulating factor (G-CSF).

18. The homomultimeric fusion protein of claim 17, wherein the homomultimeric fusion protein is a dimeric G-CSF fusion protein.

19. The homomultimeric fusion protein of Claim 15, wherein the member of the GH supergene family is EPO.

20. The homomultimeric fusion protein of any one of Claims 19 or 40, wherein the multimeric fusion protein is a dimeric EPO fusion protein.

21. The homomultimeric fusion protein of any one of claims 15-16, wherein the member of the GH supergene family is selected from the group consisting of: growth hormone, alpha interferon, beta interferon, gamma interferon, GM-CSF, IL-11, TPO, SCF, and Flt3 ligand.

22. The fusion protein of any one of claims 15, 16, 40 or 41, wherein the peptide linker is SerGly.

23. The fusion protein of any one of claims 15, 16, 40 or 41, wherein the peptide linker is $Ser(GlyGlySer)_n$, wherein n is 1 to 7.

24. A purified fusion protein according to any one of Claims 1 or 2, wherein the purified fusion protein is dimeric and is essentially free of monomeric fusion protein.

25. The purified fusion protein of claim 24, wherein the soluble protein is selected from the group consisting of G-CSF, EPO and interleukin-11.

26. A method of producing a fusion protein according to any one of Claims 1, 2, 15, 16, 20 or 41, comprising:

a. transfecting or transforming a host cell with at least one nucleic acid encoding an immunoglobulin domain and a soluble protein selected from the group consisting of a growth factor, a cytokine, and an active variant thereof;

b. culturing the host cell; and

c. harvesting the fusion protein expressed by the host cell.

27. The method of claim 26, wherein the nucleic acid further encodes a peptide linker.

28. A nucleic acid encoding the fusion protein of any one of Claims 1, 2, 15, 16, 40 or 41.

29. A host cell transfected or transformed with the nucleic acid of claim 28, enabling the host cell to express the fusion protein.

30. The host cell of claim 29, wherein the host cell is a eukaryotic cell.

31. The host cell of claim 30, wherein the eukaryotic cell is a mammalian cell.

32. A method of purifying the fusion protein according to any one of Claims 1, 2, 15, 16, 40 or 41, comprising:

a. obtaining a composition comprising the fusion protein; and

b. isolating the fusion protein from contaminants by column chromatography.

33. The method of claim 32, wherein the fusion protein is isolated from contaminants by size-exclusion chromatography.

34. A method of treating a condition treatable with a member of the Growth Hormone (GH) supergene family, comprising administering an effective amount of the fusion protein of any one of Claims 1, 2, 15, 16, 40 or 41 to a patient in need thereof.

35. The method of claim 34, wherein the fusion protein is a G-CSF-Immunoglobulin fusion protein and wherein the condition is a deficiency of blood neutrophils.

36. The method of claim 34, wherein the fusion protein is an EPO-Immunoglobulin fusion protein and wherein the condition is a deficient hematocrit.

37. A pharmaceutical composition comprising the fusion protein of any one of Claims 1, 2, 15, 16, 40 or 41 in a pharmaceutically acceptable carrier.

38. The fusion protein of one of Claims 1 or 2, wherein the soluble protein is erythropoietin (EPO).

39. The fusion protein of Claim 1, wherein the soluble protein is selected from the group consisting of alpha interferon, beta interferon, gamma interferon, omega interferon and tau interferon.

40. A homomultimeric fusion protein, comprising two or more copies of erythropoietin joined by at least one peptide linker, wherein the peptide linker is not Gly_{1-7}.

41. A multimeric fusion protein comprising two or more different members of the Growth Hormone supergene family joined by at least one peptide linker, wherein the members of the Growth Hormone supergene family are selected from the group consisting of growth hormone, prolactin, placental lactogen, erythropoietin (EPO), thrombopoietin (TPO), interleukin(IL)-2, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-10, interleukin-11, interleukin-12 (p35 subunit), interleukin-13, interleukin-15, oncostatin M, ciliary neurotrophic factor, leukemia inhibitory factor, alpha interferon, beta interferon, gamma interferon, omega interferon, tau interferon, granulocyte-colony stimulating factor (G-CSF), cardiotrophin-1, macrophage colony stimulating factor, Stem Cell Factor and flt-3 ligand.

42. The method of Claim 26, further comprising purifying the dimeric fusion protein from monomeric fusion protein.